FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

OCTOBER 13 - 15, 2004

ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF PLANT-INCORPORATED PROTECTANT (PIP), SPECIFICALLY THOSE BASED ON PLANT VIRAL COAT PROTEINS (PVCP-PIPS)

WEDNESDAY, OCTOBER 13, 2004

VOLUME I OF IV

(Morning session)

Located at: Holiday Inn - National Airport
2650 Jefferson Davis Highway
Arlington, VA 22202

Reported by: Frances M. Freeman, Stenographer

C O N T E N T S

DR. ROBERTS: Good morning. And welcome to the October 13th meeting of the FIFRA Scientific Advisory Panel.

The topic that we're going to address in our session over the next couple of days are issues associated with deployment of a type of plant incorporated protectant, specifically those based on plant viral coat proteins.

The SAP staff has assembled an outstanding, truly outstanding panel of experts, I think, to address questions that the agency are posing on this topic.

I would like to begin today's session by introducing the panel. Let me do so by starting on my left and we'll just kind of go around the table clockwise. Among the panel members I would ask each to state their name, their affiliation and their area of expertise. Beginning with Dr. Melcher.

DR. MELCHER: I'm Ulrich Melcher from Oklahoma State University, in biochemistry and molecular biology. I'm a plant virologist with expertise in recombination and bioinformatics.

DR. SHERWOOD: John Sherwood, Department of Plant Pathology, University of Georgia, plant virology, cross protection and epidemiology.

DR. ZAITLIN: I'm Milt Zaitlin, professor of Meritis of plant pathology, Cornell University, Meritis director of the Cornell biotechnology program.

My research field was plant virology. In the latter parts of my research career we stumbled on another way of making plants resistant to virus, that is, using replicase genes.

DR. FALK: I'm Bryce Falk. I'm from the University of California at Davis, a plant virologist, and my primary expertise is in virus biology and epidemiology.

DR. ALLISON: My name is Richard Allison. I'm from Michigan State University where I'm a plant virologist with an interest in the risk assessment of genetically modified plants and particularly the recombination of RNA viruses.

DR. HAMMOND: I'm John Hammond. I'm with USDA agricultural research service. I'm a plant virologist

with expertise in plant virus detection, characterization, transgenic resistance and risk assessment.

DR. TEPFER: I'm Mark Tepfer. I work at the National Institute for Agronomic Research in France. It is sort of the
French equivalent of USDA. I have worked on virus resistant transgenic plants since the middle of the 1980s and bio safety questions related to that.

DR. COOPER: Ian Cooper with Natural Environment Research Council of the United Kingdom concerned with plant viruses, how they spread, what the consequences are and laterally the risks of genetically modified plants.

DR. STEWART: Dr. Neal Stewart, University of Tennessee. I work with transgenic plants, mainly looking at gene flow introgression and the consequences in ecological systems.

DR. NAGY: My name is Peter Nagy. I'm from University of Kentucky. My major expertise is in mechanism of virus, recombination and replication and the emergence of new viruses.

DR. BUJARSKI: I'm Jozef Bujarski from Northern

Illinois University, Department of Biological Sciences. I'm a plant virologist interested in studying bio RNA recombination and replication.

DR. STARK: I'm John Stark from Washington State University. I'm an ecotoxicologist and I work in risk assessment, particularly of pesticides.

DR. GENDEL: I'm Steve Gendel. I'm with the FDA at the National Center for Food Safety and Technology in Chicago. My expertise is food safety in biotechnology and bioinfomatics.

DR. ISOM: I'm Gary Isom, professor of Toxicology at Purdue University. My area is neurotoxicology and research interest in neuro degeneration.

DR. PORTIER: I'm Ken Portier, a statistician with the Institute of Food and Agricultural Sciences at the University of Florida with interest in statistical issues of risk assessment.

DR. HEERINGA: Steve Heeringa, the University of Michigan. I'm here as a permanent member of the FIFRA SAP panel. I'm a biostatistician.

DR. ROBERTS: I'm Steve Roberts. I'm professor of Toxicology at the University of Florida. It is my pleasure to chair today's session.

Our designated federal official for today's session is Mr. Paul Lewis. I would like to welcome Paul and ask him if he has any comments or announcements for today's session.

MR. LEWIS: Thank you, Dr. Roberts. And thank you for agreeing to serve as chair for this three day meeting of the FIFRA Scientific Advisory Panel.

I also want to again thank all the members of the panel that have spent time preparing for this meeting and looking forward to upcoming deliberations that we're going to have over the next three days and the time they have at this meeting today and the subsequent report writing process taking into account their busy schedules.

As the designated federal official for this meeting, I serve as liaison between the panel and the agency. I'm also responsible for ensuring provisions of the Federal Advisory Committee Act are met.

The Federal Advisory Committee Act was

established in 1972 for a system of governing the creation, operation, termination of executive branch advisory committees.

The FIFRA SAP is subject to all requirements of the Federal Advisory Committee Act. These include open meetings, timely public notice of meetings and docket availability. That's through the Office of Pesticides Program's public docket system and the E docket system.

As the designated federal official for this meeting, a critical responsibility is to work with appropriate agency officials to ensure all appropriate ethics regulations are satisfied.

In that capacity, panel members are briefed provisions of the federal conflict of interest laws.

Each participant has filed a standard government financial disclosure report And I along with our deputy ethics officer for the Office of Prevention of Pesticides and Toxic Substances and in consultation with the Office of the General Counsel at EPA have reviewed the report to ensure all ethics requirements are met.

A sample copy of this form is available on the

FIFRA SAP web site.

The panel will be reviewing challenging science issues over the next several days. We have a full agenda of topics for discussion. And the meeting times are approximate, thus may not keep to the exact times as noted to panel discussions and public comments.

We strive to ensure adequate time for agency presentations, public comments be presented and panel deliberations.

For presenters, panel members and public commenters, please identify yourself and speak into the microphones provided since the meeting is being recorded.

In addition, a transcript will be available for this meeting in approximately 2 weeks.

Copies of presentation materials and public comments will be available in the Office of Pesticides Program docket in about two to three days and also available in the E docket system.

For members of the public requesting time to make a public comment, please limit your remarks to 5

minutes unless prior arrangements have been made. And for those who have not preregistered, please approach myself or the FIFRA SAP staff sitting behind me to register to speak at the public time this afternoon.

As I mentioned previously, there is a public docket for this meeting. All background materials, questions to the panel by the agency and other documents related to the SAP meeting are available on the docket.

In addition, the background documents and subsequent materials are available on the EPA SAP web site.

The meeting agenda lists the contact information for receiving that material either through the SAP web site or through the Office of Pesticides Program docket.

At the conclusion of the meeting, the SAP will prepare a report as response to questions posed to the agency, background materials, presentations and public comments this. This report serves as the meeting minutes basically summarizing the panel's comments during the course of our discussion. We anticipate the meeting minutes will be completed in approximately 6

weeks.

I want to again thank the FIFRA SAP members, the ad hoc members, my colleagues at EPA and members of the public for being involved in the upcoming meeting we'll have in the next three days. I'm looking forward to some very interesting and challenging discussion.

Thank you, Dr. Roberts.

DR. ROBERTS: Thank you, Paul. I would like to welcome to today's session Mr. Joe Merenda, who is the director of the Office of Science Coordination and Policy from EPA. Welcome, Joe.

MR. MERENDA: Thank you, Dr. Roberts. It is my pleasure at this point at the beginning of the meeting to welcome all the panel members as well as members of the public to this session. And particularly to thank the panel members for volunteering to take the time from your busy schedules to serve to provide this advice to the Environmental Protection Agency.

EPA's commitment to sound science is very heavily based upon getting rigorous independent external peer review of the issues that it has to deal with in

making its regulatory decisions. And among other forums, the FIFRA Scientific Advisory Panel is a key area or key mechanism by which EPA for its pesticide programs in particular obtains that kind of external review.

So we very much value your service and we look forward to your participation.

In most of these meetings, my office, the Office of Science Coordination and Policy, plays the role of convener and process facilitator for the panel meetings.

In this instance, we have a little bit of a broader role because my office also is responsible for some of the activities that the Office of Prevention Pesticides and Toxic Substances carries out in biotechnology.

Members of my staff are part of the presentation panel here today. I'm particularly interested not only from the process of having a good panel, but also from the output in this one.

Unfortunately, from my schedule, I won't be able to be with you for the entire meeting , but I will try and spend as much of the time over the next 3 days as I can

with you.

Again, thank you and welcome.

DR. ROBERTS: Thank you very much. We're going to spend much of the morning listening to some presentations by the agency. I would like to welcome Mr. Dennis Szuhay who is the chief of the Microbial Pesticides Branch of the Office of Pesticides Program, and other agency staff will be making some presentations this morning.

Welcome.

DR. SZUHAY: Thank you, Dr. Roberts. Again, I want to echo Joe Merenda's comments about welcoming you all, thanking you for your service over the next 3 days.

Most importantly, I'm looking forward to a very robust discussion of the issues before us this week, because it is my division and my branch, specifically, that will be having to work with your suggestions, deal with our management in terms of figuring out what the best road is should we choose to do some regulatory option with these particular organisms.

And it will be a very interesting discussion. I

look forward to all of your participation throughout the week.

And I also would like to add that I have had the pleasure of watching this process evolve over the last couple of months as the work group has grappled with the issues, tried to focus on which scientific questions and which issues merited the most of your attention and also in the fine tuning of the presentations that you are about to hear this morning.

So with all of this, I thank you again. I look forward to a productive week and also to a final report at the end of the whole process.

DR. ROBERTS: Thank you.

The first presentation on our agenda is one on PVCP PIPS: The Context, by Dr. Milewski.

Good morning, welcome.

DR. MILEWSKI: Good morning. It is my pleasure this morning to try to describe to you the context in which we'll be operating today.

I will try to cover some of the broader range of context which would include some of the technical things

we're going to be discussing today and also to give you a small sense of the regulatory environment in which we operate.

With that, I would like to go to my first slide. Second slide. Thank you.

This is the organization of today's presentation. Essentially, we'll be setting the stage for your discussions later today. I will be giving you your charge and discussing your context.

Dr. Anne Fairbrother from our Office of Research and Development will be talking about gene flow. Dr. Mellissa Kramer from our Office of Science Coordination and Policy will be talking about viral interactions. And then I will pop up again at the end to talk about some of the other scientific considerations to flow from the earlier presentations and the questions that you will have before you today.

I would like to briefly repeat to you the charge to this committee, which is we're asking you to provide scientific advice to assist us in our evaluation of several technical issues associated with PVCP PIPs.

Specifically, we're going to ask you to respond to a series of technical questions related to exposure and hazard considerations for PVCP PIPs.

To begin, what is a PVCP PIP. Well, PIP is an acronym for a plant incorporated protectant. That is a type of pesticide.

PVCP is an acronym for plant virus coat protein. And because it's kind of a tongue twister, we have agreed amongst ourselves that the first P would be silent. So we would say VCP PIPs. But every once in awhile when you see it up there you put in the P. Sometimes I'll be saying PVCP PIPs and sometimes I'll be saying VCP PIPs, which even as I say it you can hear the second is a little bit easier, at least I think.

Hopefully with that explanation let's try to use VCP PIPs.

What is a plant incorporated protectant? Essentially, we have defined it as a pesticidal substance that is intended to be produced and used in a living plant or in the produce thereof and the genetic material necessary for production of such a pesticidal substance.

I have underlined what I think are the 2 key ideas that we have to carry forward today. One is that when we talk about a PIP, we are talking about the pesticidal substance, which could be a protein produced or it could be another substance such as a messenger RNA and the genetic material that's necessary to produce it. In this case, for example, the DNA.

Basically, the definition of a PVCP PIP is a PIP created from the gene or a segment of the gene that codes for coat protein of a virus that naturally infects crop plants. That's the definition we'll be working with today.

Because what we'll be talking about for most of this meeting is actually risk considerations, risk issues, I did want to put in one slide that reminded all of us that we see benefits for PVCP PIPs.

We see them as an effective means of controlling virus infection. And the consequences of that could be higher yield for the farmer. It could be reduced use of chemical pesticides to control insect vectors which would

be an environmental benefit that the agency would value.

In some cases, we see it as being the only option as a means of controlling viral diseases in certain crops.

Now, to switch to the technical questions we'll be talking about at this meeting, they are broadly divided into 3 groups. One of them is gene flow. The other, viral interactions. And then the third, these other scientific considerations that kind of tail along from the other two.

For gene flow, EPA is seeking your assistance to better understand circumstances in which the flow of PVCP PIPs from transgenic plants to wild or weedy relatives occurs and also the potential for adverse impacts from such gene flow.

Obviously, just because gene flow may occur does not automatically follow that there would be an adverse impact associated with that gene flow.

Then we're going to ask you to identify and evaluate conditions that might minimize gene flow should such minimization be seen as appropriate.

For viral interactions, we're seeking your assistance to identify and evaluate circumstances wherein interactions between introduced virus sequences and invading viruses might be more frequent than expected to occur in natural mixed virus infections or unlike those expected to occur in such conditions.

Similarly, as for gene flow, we will ask you about conditions that might minimize such occurrences should such minimization be seen as appropriate.

The other scientific considerations we will be talking about, we're seeking your assistance in evaluating technical issues that might be associated with PVCP PIPs.

One of the important things for us is to understand the boundaries of the assumptions under which we would be operating. Then, of course, we would ask you if there are any additional considerations for minimizing risk that we have not put forth in our questions to you.

Your role in all of this is to assist EPA in better understanding specifically the degree of risk for each of the issues that we present to you. We would like to have a sense of your degree of certainty of your

estimates.

We would like to understand a little bit better what of those estimates of risk are coming from data that you have actually seen generated and which of them are hypothetical.

Assuming that there may be things in this meeting that are going to be hypothetical, we would like to get a better sense from you of the direction the science is taking based on these specific issues raised.

We would ask you to provide us technical recommendations and advice on the technical questions posed.

How will your advice be used? We would then take your advice and we would use it within the parameters established by the statutes under which we operate. I'm going to take a little bit of a detour here, it won't be very long, just to talk a little bit about those statutes.

Within the legal context, we operate under 2 statutes to regulate pesticides.

One, you probably are familiar with, the Federal

Insecticide Fungicide and Rodenticide Act. Our responsibilities under that act are to protect both the environment and to protect human health.

We also have responsibilities under the Federal Food Drug and Cosmetic Act, Section 408. In that, we're to determine safe levels of pesticide residues in food and feed.

So while most of our questions today are going to deal with environmental issues, there is a question in there that goes towards the food safety consideration that might be associated with PVCP PIPs.

FIFRA defines a pesticide as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest.

As you all know, the PVCP gene sequences can confer resistance to the virus from which it was derived and often to related viruses, to the recipient plant.

You can see that PVCP PIP falls within the definition of pesticide under FIFRA.

Now, one thing that I also should remind us all is that the United States operates under a coordinated

framework, a framework of laws and regulations that address all products of biotechnology in the United States. So EPA is just one component of a regulatory structure for virus control strategies.

We call them PVCP PIPs. That happens to be a term of art for EPA. I'm sure our colleagues from USDA would call them something slightly different. But at any rate, there are 3 regulatory agencies that play complimentary roles in oversight of these types of products.

EPA, we regulate the pesticidal functions of the particular product.

FDA is responsible for the food safety aspects of a plant that have been modified to express PVCP PIP except for the PVCP PIP itself, which is within EPA's bailiwick.

And USDA addresses the plant pest risks presented by these particular types of products.

The history of PVCP PIPs at EPA is actually quite a long one. Probably in regulatory terms, not all that long, but I have been involved with this particular

issue since the late 1980s.

In 1994, EPA put forth a proposal to exempt PVCP PIPs. We had 2 alternatives in that proposal. We proposed either to exempt all of them or as an alternative to exempt some of them depending upon potential for weediness. We actually put forth some criteria that would help people determine whether their particular PVCP PIP had weediness potential.

We did not finalize that in 1997. We went out with a supplemental request for additional comment to exempt PVCP PIPs. Then in 2001, once again we asked the public to help us with this particular issue.

So there's been quite a number of times that we have gone to various groups to ask for their assistance.

I should say that we actually went to the FIFRA SAP back in 1992 and in 1994 before the proposal came out for their assistance in helping us craft the proposal. So it is actually quite a long history of assistance for us.

One of the reasons that we have had had to be going back to the public and then back to the SAP is

because we have received such a wide range of comments from the public. Some of them would support the exemptions. Others were opposing it.

I have put down some examples of the types of comments that we received. As you can see, if you look under support for exemption proposal, there is one that says, wild species are generally already resistant or exhibit a high degree of tolerance to infection.

The second comment was, since viral coat proteins do not act in a toxic manner, all viral coat proteins should be exempt.

Then we have comments opposing exemption proposal.

The sexual transfer of engineered virus resistance would readily confer an advantage to weedy populations.

The second comment. Genetically engineered virus resistant crops present serious ecological risks. One, new viral strains may emerge through recombination and transcapsidation.

Now, these comments actually came in response to

a 1994 proposal to exempt. You can see that we're still asking many of those same questions of you today.

This has been a fairly long period of discussion of these issues.

The next slide shows a more recent set of comments. This is actually from the 2000 National Research Council report entitled Genetically Modified Pest Protected Plants Science and Regulation. This report raised a number of questions which again we're still discussing.

For example, they raised questions about the potential for gene flow from transgenic to weedy relatives.

They also had suggestions that transgenics could be constructed with mitigated controls to reduce potential for viral interactions. Again, something that we'll be talking about during this meeting.

I have given you just a very, very brief overview. Sort of a 70,000 foot overview of the context for this. We have had a very long history involving complicated issues, but I would emphasize that we're

focusing very narrowly in this meeting. We're asking you to provide scientific advice to assist EPA in its evaluation of several technical issues associated with PVCP PIPs. We're asking you to focus on a series of specific questions posed by us.

I think that's my last slide.

DR. ROBERTS: Thank you. The next presentation is by a veteran of some SAP meetings of recent years, Dr. Fairbrother, on gene flow in viral coat protein transgenic plants.

DR. FAIRBROTHER: Thank you very much. The purpose of this presentation and the next one is to give some overviews of the technical details and information that we're going to be discussing over the next 3 days.

To kind of get us all on the same playing field, to provide some definitions and get the conversation started.

This presentation we're going to be talking about gene flow. For the purposes of our discussion here, we're going to define gene flow as the movement of genes including transgenes, of course, but also natural genes

from crops to weeds and wild relatives or to other crops.

This is just the movement of genes. And introgression, then, is when the gene becomes fixed in the recipient population. So two definitions, differences between gene flow and introgression.

A lot of the talk in the materials come from several reviews and other papers that were presented to you in the background documents that you were given. The three that you see here in red were also given to you as the entire review material as well and we're lucky enough to have a couple of the authors here with us on the panel to continue with the discussions and provide additional detailed information.

We do know that gene flow is something that can occur every year. Every time plants pollinate or produce seeds we can get movement of genes.

Introgression on the other hand is something that can happen either quickly or can take multiple years, multiple introductions. And this certainly depends upon the particulars of the plant system that we're looking at. Rates of pollen and seed dispersal are particularly

important as are the relative sizes of the population of the donor plants and the recipient plants.

So the relative size in this case of potentially crop systems versus the recipient wild or weedy plants.

Selection pressures that are on the recipient plants and also reproduction times. So do plants reproduce every year or is it multiple years for each generation.

There has been quite a lot of discussion of this in the literature and many concerns have been raised, some of which have been studied and some of which are still speculative. But some of those that have been talked about the most are here on this slide.

And that looks at potential effects on the recipient plants of the movement of genes into those plants, particularly whether this is going to increase or decrease the fitness of those plants.

Potential effects on plant communities then can emerge from this as we have changes in competitive advantages of the plants that have received the new genes and changing the potential of extinction risks either of

the plants that have received the genes or those that they interact with.

There is also the potential for effects on genetic diversity in the ecosystem. Genetic swamping is one such example of the formation of hybrids that then eliminate a already existing taxon because of the interbreeding that has occurred.

There is also the potential for indirect effects, not just within the plant community, but also on other parts of the ecosystem related to herbivore and the food chain.

There is a number of lessons that have been learned about the movement of gene flow. We'll review some of those briefly here. Some of this is information that's been learned from looking at the movement of natural genes as well as some studies more recently on transgenes.

Allison Snow in her recent discussions in Nature Biotech has suggested that transgenes can act like conventional genes in terms of dispersal and introgression rates. That there may not be anything particular about a

transgene that would change the rate of gene flow.

Gene flow can be widespread and can happen regularly year after year. And as a result, you can see either increases, decreases or no changes in fitness of the recipient plant populations depending upon the genes that are transferred.

She goes on to say that we need to remember that the first generation hybrids, the F1s, may be sterile, but this does not mean that they cannot propagate. They can continue to transmit genes widely through asexual reproduction, of course, either through vegetative propagation or through development of seeds without pollination.

Genes that are not on chromosomes can be transferred, albeit at a much lower rate than the chromosomal genes generally are.

She also points out that fitness changes occur only after the plants are released from some type of strong limiting factor that naturally is on that particular plant.

I would like to take a moment to define what

we're talking about when we keep using the term fitness. It has come up a number of times already and will continue to do so throughout this talk and I'm sure the rest of today.

Ecological fitness is the relative ability to contribute offspring to the next generation. It can be measured either on a population level by looking at the finite rate of increase of that particular population or at the individual level by measuring the number of offspring that individuals produce.

Plants have many different kinds of fitness strategies, some of which are increasing number or size of seeds that are produced, perhaps having a faster rate to reach maturity, greater resistance to stress such as draught, temperature extremes, disease, including viral diseases, parasites and so forth and also differences in soil properties.

There is probably many more that you can think of to put on this list, but these are some of the things that plants do in order to increase their fitness.

What are some of the lessons learned that we

have seen over the years in terms of gene flow first?

Ellstrand, et al., reviewed in 1999 13 of the most important conventionally bred crops in the world to see how many of those have demonstrated gene flow to wild relatives. And noted that 12 out of those 13 have shown gene flow, of which 7 can be said to have introgressed into the wild populations.

He has since updated that review in his book, Dangerous Liaisons, published last year, and has shown that 22 out of 25 of the largest crops in the world have evidence of gene flow.

In the U.S., that would be about 55 percent of our crops. He has pointed out 11 out of 20 of the crops grown here.

Now, both Ellstrand in his recent book and also Allison Snow have pointed out that there are many other crops that are of lesser world importance that also have been shown to exhibit gene flow.

Along those lines, Dr. Stewart has pointed out that there are tens of thousands of potentially occurring natural hybridizations that can occur amongst plant

communities. But many, many of those have not been well documented.

There has been 165 that have been confirmed. And he suggested that of those, 65 have been sufficiently documented to be able to say with certainty that gene flow has occurred.

It is difficult at times to separate out hybridizations that are genetically based from evolutionary convergence where you can see plants that look similar, look like they might be hybrids, but are genetically distinct.

Over the last 10 years or so with the development of new genetic techniques particularly looking at different types of DNA polymorphisms, we have a much increased ability to be able to determine these degrees and types of relatedness among plants and learning quite a bit more about the relationship of plant genetics to plant morphology.

Dr. Stewart in his review also reviews one of the more well known examples of hybridization in a natural system. That's between 2 species of iris they find in the

southeast U.S., iris fulva, which grows in salt marshes, and iris hexagona, which is a related plant and grows nearby, but in more freshwater swamps.

These two can exchange genes and have been shown to do so over distances of about 10 to 25 kilometers. So a fair distance. You can end up with intermediate hybrids which have different fitness characteristics if you look at their ability to survive in the brackish water swamps.

Those that are like the male parent iris fulva tend to have intermediate or higher fitness, whereas those that are like the female parent, the hexagona have intermediate or the same fitness.

Also, this system has shown the development of a new taxon that is the hybrid between the two where the genes have become fixed and the hybrids can cross with each other without needing to backcross and produce similar species.

So what this has taught us then is that there can be local geographic formation of what are known as hybrid swarms where you have either the plants that can

interbreed in the F1 and F2 generations or interbreed as backcrosses with the parents. The gene flow can occur beyond the range of the original hybridization zone and actually occur over large distances with a formation of a new stabilized taxon.

So continuing then, Dr. Stewart has pointed out that introgression of transgenes from GM crops to wild populations can occur, but is more difficult than from wild plants to crops.

He also suggests that there may be -- that the linkage to domestication alleles can impose a barrier to gene movement and that domestication genes reduce ecological fitness.

So that crops themselves are less fit than their wild relatives.

Now, Dr. Norm Ellstrand in his book has also suggested that, with rare exceptions, transgenic traits in plants are almost always dominant traits. And this really makes sense. And if we're gong to all of the effort to develop a particular gene to put into a plant for a reason, you want it to be expressed all the time. So

putting it as a dominant trait would be an appropriate approach to take.

However, what this means then is that transgenic hybrids will always express the trait. If you get gene transfer to a wilder weedy relative, they will be immediately subject to selection pressures.

Traits that distinguish cultivated plants on the other hand are usually recessive alleles, and so the plant needs to be homozygous for those alleles. Although when they are, they do appear to have a major fitness effect and can be modified by other alleles that have minor fitness effects.

So for example, if you have a trait that is looking for development of big seeds, it can also be modified by other traits that will allow those seeds to mature more quickly.

There are those that have suggested that the use of mathematical models might be an appropriate way to look at the possibilities and likelihood of gene transfer and introgression and the subsequent effects.

Haygood, et al., published a paper last year

that using such a model -- or they argued that at even very low transmission rates of the transgenes to wild populations you will eventually result in fixation of the gene, even if this may take decades that it will happen.

Now, Dr. Stewart has argued that Haygood's predictions and his model may be based on some assumptions that one can take, can argue with about the basis of the model. But it does continue to raise the question that we all need to address about what is the ecological significance of even very low levels of gene flow.

Dr. Ellstrand has also pointed out that rates of mating of crops with wild relatives are no different for transgenic crops than for conventional crops suggesting that there is nothing about transgenes that would make gene flow be any different with the exception of those crops that are engineered to reduce fertility.

However, there are others who are beginning to point out that this may not always be true. And there was a paper published a couple years ago by Joy Bergolson (ph), for example, who has shown that an herbicide resistant transgene was transferred more frequently in a

rabbit opsis than the naturally occurring herbicide resistant mutant.

I think that still may be open to question.

Taking a look, then, at how we categorize the potential risk of crop to wild introgression by transgenes, these are some suggestions that Dr. Stewart had put forth in his paper. That we need to look particularly at colocation with wild relatives, if there is any evidence for crop to wild gene introgression from normally occurring genes and also the degree of genetic differentiation between the crop and its wild relatives.

So looking now more specifically at gene flow concerns for the PVCP PIP crops, Dr. Tepfer has reviewed some studies and has shown that there is a significant negative impact of virus infection on growth, survivorship and reproduction of some plants. As you recall, when we started out this discussion, Dr. Snow's paper had pointed out that in order to have an effect, a transgene must affect something that is a controlling factor on plant populations.

So this work is looking at the fact that virus

infection can be a controlling factor on some plant populations.

So, therefore, we do need to be concerned with the potential for increased weediness or competitive advantage of plants with virus resistance genes.

Here is an example of such a situation with a barley yellow dwarf virus. This is a luteovirus that causes significant amount of crop damage. Conventional breeding has not been able to develop a resistance or tolerance strain.

Some virus strains move via aphid transmission into wild hosts such as wild oats, squirrel tail grass and other such species which also have no natural resistance and will show signs of infection.

Wild oats are known to be a agronomic weed in many cultivated cereal crops and have been introduced into western United States, particularly in California where it already outcompetes many of the native grasses.

Cultivated oats will hybridize really with wild oats at a relatively high rate.

The hypothesis then is that fitness,

particularly growth and reproduction, could be enhanced with a PVCP PIP against this virus, and if wild oats were to receive that.

If that were the case, that release from the virus infection could increase a competitive advantage of the wild oats species, increasing their weediness in cereal crops and also increasing their invasiveness of grass lands in places such as California. Although this might occur only in the absence of other mitigating environmental factors, it certainly is a possibility.

On the other hand, we have an example here of where a virus infection is not a controlling factor in a plant population and transmission of a PVCP PIP to that wild plant may not have an ecological consequence.

This is an example presented by Dr. Tepfer of the sea beet which is the progenator of all of our cultivated beets and is susceptible to the beet necrotic yellow vein virus.

This virus is absent in brackish water environments where the sea beet naturally occurs, because

the fungal vector that transmit the virus cannot tolerate the salty soils of that environment.

Therefore, receipt a transgene that confers resistance to this virus into the sea beet would not have any selective advantage or disadvantage because of the lack of the vector for transmitting the virus.

Some of the lessons that we have learned from all of this are that features that increase the likelihood of gene flow are sexual compatibility between the donor and recipient plants, that they are grown in the same vicinity and have overlapping flowering times.

If the F1 hybrids persist for more than 1 generation, it will significantly increase the likelihood of gene flow, and, particularly, if they are fertile and can also backcross with the parent plants.

Features that will increase the likelihood of introgression are dominance of the gene and also that it confers a selective advantage such as we were just talking about.

The absence of association with deleterious crop alleles or trait will also increase the likelihood of

introgression, as well location on a shared genome between the donor and recipient plants.

Some plants, as you know, have multiple genomes and their close relatives may not have all of the same genomes. So location on a particular genome can be very important as to whether a gene will cross and introgress.

Similarly, location on a homologous chromosome and particularly on non rearranged chromosome.

So taking that, then, some of the approaches to decrease the likelihood of gene flow and introgression, perhaps not to completely eliminate them, but at least to decrease the likelihood of doing so is placement on nontransferred chromosomes, linkage to deleterious crop alleles or traits. So if the gene does get transferred, the fitness of the recipient plant is also decreased.

Insertion into maternally transmitted organelle DNA such as in chloroplast. Induced sterility of the transgenic plant to decrease pollen formation and development or germination of seeds. And deployment in areas where crops have no known wild relatives.

In conclusion, then, we have seen that gene flow and gene introgression can occur between crops and their wild and weedy relatives, although the likelihood and consequences can vary greatly depending upon crop species, recipient species and the genes transferred.

Questions certainly remain about how to characterize the potential for risks of crops with PVCP PIPs.

And that is the topic of much of our discussion for this afternoon.

DR. ROBERTS: Thank you, Dr. Fairbrother.

I would like to pause for a moment and give the panel the opportunity to ask you any questions they might have, and also I guess extending back into Dr. Milewski's presentation.

Are there any questions from panel members? Yes, Dr. Zaitlin.

DR. ZAITLIN: I would like to make a point of clarification on the definition of PVCP PIP as given by Dr. Milewski. She says that the definition is it controls virus infection.

I think that's really very narrow. Because what you are really interested in is in the control of virus disease. Because some of these plants actually can support virus replication. They can inhibit the disease. They can prevent movement or suppress the symptoms.

DR. ROBERTS: Dr. Stewart.

DR. STEWART: I guess I would like to point out that while I agree with many of the points that were in the -- probably most of the points that were in the presentation, many of the citations came from non peer reviewed literature, commentaries and book chapters and books which often express more opinion than material from scientific data.

That's just a point of clarification, I think.

DR. ROBERTS: Okay. Dr. Gendel.

DR. GENDEL: Sort of as a follow up to that, when I was researching the background information for this, I found it difficult to obtain the information on the original SAP panels and the original policies. The background data and the SAP results, I guess they go back to the days before everything was put on the web.

So other than summaries of what all the conclusions were, I couldn't find the data that went into making those conclusions and the deliberations. It would have been interesting to see the discussions that took place at that time and how much was peer reviewed literature and how much was not.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: It's maybe a rather minor sort of clarification, but in one of Dr. Fairbrother's slides, there was one point where she was commenting on the review article I wrote a couple years ago. On the slide it said, viruses are controlling factors for some plant populations. In her oral presentation she said could be.

I think it is very important that my particular opinion on that is that it could be. It is a purely hypothetical case at the moment. Please don't take what is written on the paper as my point of view on that.

It is a very interesting question of science, but we don't know the answers yet.

DR. ROBERTS: Dr. Falk.

DR. FALK: Maybe my comment might be similar regarding to one of the slides that you showed regarding the article written by Dr. Tepfer where I think you said that studies have shown significant negative impacts and you mentioned populations and you mentioned a few species.

Was that correctly reported in your article, Dr. Tepfer?

DR. TEPFER: I think that those are, yes, examples where there were fitness components that were affected by virus infection in those plant species.

DR. FALK: In natural population.

DR. TEPFER: Not all of those are natural populations. The wild squash was all under experimental conditions. For some of the brassicas -- yes, the others were under natural conditions.

DR. ROBERTS: Other questions?

If not, let's move ahead to the next presentation. By the way, Dr. Fairbrother, you knew that -- I sympathize with the difficulty of summarizing the results of many of the members of the panel who are here who can always take issue with nuances of your

presentation.

That was a tough job. You knew you were going to get a few comments back on that.

Let's go ahead and move on with the next presentation.

DR. KRAMER: I'm going to follow up Dr. Fairbrother's discussion on gene flow issues to try to set the stage for the questions we're posing on vital interactions.

As was the case with Dr. Fairbrother's presentation, I, too, am faced with the task of summarizing many research results from scientists here in the room.

I don't think I need to go over this for most people. But just to set the stage, I wanted to provide the basics of virus and the virus infection cycle.

First, the virus enters the plant through a mechanical breach of the cell wall. At that point, it may shed its protein coat and be able to replicate within the cell.

Movement proteins are needed to modify the

plasmodesmata that would allow the virus to cross the cell wall and then spread throughout the plant, at which time it would become available for a transmission to a new plant.

In terms of transgenic virus resistance, many different types of transgenes have been used experimentally to confer resistance. Most notably, of course, plant viral coat proteins, but also viral replicase, genes movement proteins, nuclear inclusion genes as well as a number of non viral sequences.

However, plant viral coat proteins are the most common and are the sole topic of the discussion today, I would just like to point out that this has been a topic for discussion for many years now.

The first report of a plant viral coat protein transgenic plant was published in 1986.

So just again some basic information about plant viral coat proteins. They encapsidate the viral nucleic acid and are important in nearly every stage of virus infection from replication to movement throughout an infected plant and also from transport from plant to

plant.

There are a number of mechanisms proposed for how coat protein mediated resistance works. A lot of research is still being conducted in this area.

There are basically two major categories of mechanisms, though. One would be protein mediated. That's -- it is believed to be protein mediated because the level of protection correlates strongly with the level of MRNA and protein accumulation within the plant.

It is thought that that works through the transgenic coat protein actually blocking the uncoating of virions upon their entry into the cell.

However, it was discovered that it is not always the case that there is such a correlation. In fact, at times it was discovered that there was no correlation between the level of MRNA and the level of protection conferred to the plant.

Therefore, it was hypothesized that there was a nucleic acid mediated mechanism for virus resistance. And further research has shown that that's likely to be due to post translational transgene silencing which suppresses

expression of the transgene and any accumulating viral RNA that shares sequence homology with the transgene.

To set the stage for really what we're considering is the baseline for our discussions today in transgenic plants, I wanted to go over what we know about mixed virus infections.

They occur when viral genomes from different strains or species simultaneously infect the same plant. They can be extremely common among certain types of plant and certain plant populations.

In rare cases, mixed virus infections have been implicated in adverse agricultural or environmental events. For example there is a case of cassava mosaic disease in Uganda which was thought to be due to either sequential or simultaneous occurrence of recombination, pseudo recombination of synergy.

This is worth pointing out because the fact is that in VCP transgenic plants, every infection of a virus other than the one from which the transgene was derived is essentially a mixed infection with respect to the VCP gene itself, which brings us then to really the critical

question that is overarching all of the questions we are asking the panel today in this area.

It is, Are the risks associated with virus interactions in VCP transgenic plants greater in degree or different in kind than in natural mixed infections?

Here are some issues to consider. This is really an outline of the rest of my talk. For each of the 3 types of virus interactions, recombination, heterologous encapsidation and synergy, I want to go through what we know about its occurrence under natural conditions, what we know about its potential to occur in VCP transgenic plants and then ways that have been studied or hypothesized to reduce the frequency of these events if it were gene warranted.

Then I want to focus really on the field evaluations that have been done because of their crucial significance for evaluating the ecological significance of these events. And then bring us back to the critical question here, dividing it into 2 parts.

The first, is the frequency of virus interactions in PVCP transgenic plants different than in

natural mixed infections, either higher or lower. And second, is the nature of the virus interactions in PVCP transgenic plants different than in natural mixed infections.

Starting now with recombination. Recombination means that segments from different parental molecules may form chimeric molecules. The mechanism in RNA viruses is thought to be template switching of the viral replicase during replication.

Under natural conditions, recombination very rarely leads to new viable viruses. That's the case because a virus must recombine such that it is competent in all stages of virus infection. That can be very difficult to do.

Nevertheless, it is important to point out that recombination has still been thought to play a significant role in virus evolution in a number of virus groups. It is more likely among closely related viruses that can undergo homologous recombination.

It's also important that both virus-virus recombination and virus-host recombination can occur.

That is, you can get actual incorporation of host genes into viruses.

So recombination in transgenic plants with virus trangenes. When a virus infects a transgenic plant, those nucleic acids may become available for recombination with the host transgenes. And lab experiments have indeed shown that such recombination can occur.

These experiments have almost always been done under high selection pressure. That is, the only way that a competent virus could be produced at all is through recombination. Such high selection pressure isn't expected to occur in the field where the recombinant virus would be competing with the parental viruses from which it came.

Therefore, the ecological significance of these experiments is unclear beyond showing that such recombination may in fact occur.

A number of ways have been investigated for reducing the frequency of recombination. I listed a few here. One, removal of the 3 prime untranslated region necessitating a double crossover to produce a viable

recombinant.

Secondly, excluding any replicase recognition sites or other known recombination hot spots in the construct.

Third, reducing the extent of shared sequence similarity, for example, through the introduction of point mutations.

Fourth, using the smallest viral fragment possible that would give the smallest target for recombination while still allowing virus resistance to be conferred.

And finally, the insertion of GC rich sequences downstream of AU rich sequences has been shown to occur in at least one virus system, although its applicability broadly has not been demonstrated yet.

Moving now to heterologous encapsidation. This means that the coat protein subunits of one virus may surround the nucleic acid of a different virus.

Under natural conditions, it is known that this can affect virus vector interactions, which is perhaps not surprising given that the coat protein does play a

prominent role in interactions with vectors.

Among some plant viruses, it can, in fact, be a very regular occurrence. There may indeed be viruses that require heterologous encapsidation for transmission because they don't produce any coat protein of their own at all.

Therefore, it can be a very natural part of virus epidemiology. As (ph) recombination, it's more like to occur among closely related viruses.

I think it is important to expand on the situation under natural circumstances to point out that there is usually limited environmental concern due to heterologous encapsidation for a number of reasons that I would like to go through.

First, vector specificity is often determined by the coat protein, but often only partially determined. Therefore, the encapsidation by an unrelated coat protein may not be sufficient to allow a new vector to transmit it.

Secondly, vectors may carry a heterologously encapsidated virus only to plants it already infects.

You could imagine if you had a large monoculture of a plant growing in a field, a new vector that may be able to pick it up because it contains a novel coat protein may very well be likely to only transmit it to other plants of the same type within the field.

Therefore, although heterologous encapsidation occurred, it would only be transmitted to a plant that the virus is able to infect anyway.

Finally, perhaps most importantly, once the virus replicates in a novel host, if it is able to replicate, it then becomes reencapsidated in its own coat protein and, therefore, it will not be competent to be transmitted by novel vector that put it there in the first place.

However, I think it is important to point out that one could imagine certain limited circumstances under which you might expect that there could be some environmental concern due to heterologous encapsidation.

One, a high enough frequency of heterologous encapsidation. Even if the virus then becomes encapsidated in its own coat protein in those new plants

may mean that you don't necessarily require a secondary transmission of new host plants for impact. Particularly, if you are thinking about a rare susceptible population.

Secondly, viruses are thought to exist as quasi species in which the many different types of viruses differ by few nucleotides from a consensus sequence. And the most best adapted variance may be able to rapidly evolve in a new host.

Thirdly, once in a novel host, there may be potential for exposure to new vectors that it didn't have interaction with in the plant that it came from.

So what do we know about heterologous encapsidation in transgenic plants with viral transgenes?

Laboratory experiments have been done to show that protein from VCP transgenes can encapsidate infecting viruses, even unrelated infecting viruses.

I have there, protein, when it is produced. Because as I mentioned before, the mechanism of resistance in some cases is nucleic acid mediated. In those cases, no protein may be produced, in which case heterologous

encapsidation would obviously not be a concern.

A number of ways have been hypothesized and investigated for reducing the impact of heterologous encapsidation. I say the impact because that can be done in two different ways.

One would be by reducing the frequency of heterologous encapsidation itself. The other would be by reducing the frequency of vector transmission per se.

That is the heterologous encapsidation may still occur, but that heterologously encapsidated virus would be expected to remain in the plant where it was originally infected.

Certain regions are known to affect aphid transmission specificity. A few are listed here on the slide. These have been hypothesized as good candidates that one might target in the design of a construct to eliminate them or mutate them and thereby affect aphid transmission.

A number of experiments have been done looking at PVCP gene modifications that have been shown to reduce the frequency of either heterologous encapsidation or

vector transmission. A few of those are listed there on the slide as well.

Moving then to the third type of virus interaction. Synergy. Synergy is when the disease severity of 2 viruses together infecting a plant is greater than expected based on the severity of each alone.

Under natural conditions, there are many known viral synergisms. They are more common among some viruses than others. Important for our context here today, the coat protein is less likely to be responsible for viral synergisms than other regions of the virus genome.

In transgenic plants with viral transgenes, synergy is largely an agroeconomic concern. That is that the impacts are expected to most directly affect the transgenic plant itself. Therefore, there is a high incentive for a developer to evaluate synergy before deployment because it's the efficacy of the product itself that is at stake.

If by chance there were, in fact, a product that were able to be deployed and a synergy were discovered

after deployment, farmers would likely quickly abandon the product because it would not only not achieve the goal for which it was purchased, but the farmer would in fact be worse off than before deploying this product.

A number of ways have been investigated or hypothesized for reducing the frequency of synergy. Again, constructs may be engineered to reduce the likelihood of synergy by avoiding particular transgenes known to be involved or using defective copies of genes.

Another strategy may be to stack multiple resistances within the same plant, thereby reducing the frequency of mixed virus infections and, therefore, the potential for synergy between different types of viruses to occur.

So now I want to really move from what I have been talking about which is basically what we know from laboratory experiments about these viral interactions to talking about field evaluations that are really critical for assessing what the impacts and likelihood of these types of events are.

There have been really a relatively small number

of published studies that have been done in this field, but they to this point seem to provide no evidence of adverse effects. I actually because of the small number I want to run through the major ones here.

The first is Thomas and others in 1998. This is a relatively long and large experiment looking at 25,000 potato plants, 442 lines transformed with 16 potato leaf role (ph) virus coat protein constructs.

They were exposed to field infection over 6 years. At the end of that time, they looked and found there were no new viruses or viruses with altered transmission or disease characteristics detected as you might expect if any of these virus interactions had occurred and led to a significant impact.

Fuchs and others in 1998 looked at transgenic melon and squash containing the coat protein from an aphid transmissible strain of cucumber mosaic virus. They infected the plants with an aphid non transmissible strain of cucumber mosaic virus, the idea being to see if the transgenic coat protein would be able to heterologously encapsidate this other virus strain and therefore enable

it to be transmitted by aphids.

At the end of this experiment they found there was no aphid vectored spread of the non transmissible strain.

Fuchs and others in 1999 did a very similar experiment this time looking at transgenic squash containing the coat protein from an aphid transmissible strain of watermelon mosaic virus.

Plants were infected with an aphid non transmissible strain of zucchini yellow mosaic virus. The results were a little bit different in this experiment.

In nontransgenic fields, they found there was no transmission of the zucchini yellow mosaic virus. However in transgenic plants, there actually was transmission to 2 percent of the plants in the transgenic fields.

It was thought this was likely due to heterologous encapsidation. However, it is important to point out that no epidemic of the disease developed.

Lin and others in 2003 did a little bit type of different experiment looking really at the potential for resistance to virus infection to evolve. They estimated

the biological and genetic diversity of cucumber mosaic virus isolates before and after development of transgenic squash containing the coat protein from 3 different viruses.

What they found is that most cucumber mosaic virus isolates showed no significant sequence changes between those infecting the transgenic squash and those infecting the non transgenic squash.

There was one isolate that did differ, but it was not due to recombination or selection.

Finally, Vigne and others in 2004 looked at transgenic grape vines containing the coat protein of grape family virus.

Non transgenic scions were grafted onto transgenic and non transgenic rootstocks. They were exposed over three years to grape family virus infection.

The transgenic grapevines were found not to assist in the emergence of viable grape family virus recombinants or to affect the molecular diversity of indigenous population.

So now I want to return back to what the critical questions were that I posed at the beginning. The first being does the frequency of interactions change in viral coat protein transgenic plants?

I point out at first that there is really as far as I know essentially no data on this topic. That's because it is very difficult to measure these events due to their rarity in any case.

There are some factors that suggest there may be a decrease in the frequency in transgenic systems. That is that there is generally going to be a lower concentration of cellular RNA transcripts from a transgene than there would be from an infecting virus that would reach a very high titer (ph) in an infected plant.

Secondly, assuming that the virus resistance is, in fact, working, there would be a lower concentration of infecting virus in that transgenic plant.

However, there are some factors that suggest you may in fact get the opposite case and that there could be an increase in frequency in transgenic systems. One would be usually these transgenic plants are constructed with

constitutive promoters. The cauliflower mosaic virus promotor that would cause expression to occur in all developmental stages and all tissues of the plant.

That may not necessarily be the case in all viruses that could be cell or stage specific.

Secondly, there are thought to be natural, temporal or spatial expression patterns that could be obscured in a transgenic plant. That is that in natural systems a virus may enter a plant cell wall after uncoats and replicates and moves to other cells within the plant. That virus may become reencapsidated and be unavailable for interactions with other infecting viruses.

Second part of the question is whether PVCP transgenic plants might lead to novel viral interactions. I think it is here important to point out what we really mean by novel viral interactions.

Prior to this, I had been talking about viral interactions per se. When I say novel what I mean are interactions that we would not expect to occur in a natural system. That is this particular sequences that are interacting do not exist in nature in that

combination.

Here are some examples that one might imagine could lead to such an interaction. The first one would be transgenic multi resistances. That is if you stack multiple virus resistances within the same plant or you stack virus resistance with some other trait, say an herbicide tolerance trait, you might expect that you would increase the likelihood that that product could be deployed in an area where you are not actually attempting to control the virus.

Perhaps an area where the virus is not actually infecting you may be introducing virus sequences into a system where they weren't previously.

Second would be heterologous resistance. It is known that in some cases you can, in fact, get resistance to a certain virus through incorporation of a similar type, but a different type of virus coat protein into a plant.

If any such product were ever developed, you might obviously expect you could deploy that in an area where the virus from which the coat protein was derived

did not actually infect those plants in that area.

The third would be you could imagine the use of an exotic strains coat protein. Perhaps this could be intentionally done to try to stave off the infection that's known to exist in other parts of the world in a region where that virus does not currently exist.

But doing so may, in fact, be introducing virus sequences that would be competent for recombination with a similar infecting virus that is in the area. You could have new types of novel interactions through that mechanism.

The fourth I have actually already touched upon. That is you can get expression in new cells or tissues through the use of a constitutive promoter and may leave a virus available for interaction with viruses that don't normally infect the type of cell that it does naturally infect.

Finally, this is getting at a question we'll be talking about later. It is possible to alter a coat protein gene within a construct in a myriad of ways. Our question really is how much can that be done. At what

point do you achieve something that is so unlike anything that exists in nature that you would have what you might call a novel viral interaction occurring.

Just in summary, I want to put forward now what our overarching issues are for the panel to consider. The first is are viral interactions in PVCP transgenic plants an environmental concern above and beyond what occurs naturally in mixed virus infections.

First, is there a potential for increased frequency of interactions and, second, are novel interactions, again, I want to emphasize novel, are novel interactions likely to occur and have any adverse environmental impacts.

Secondly, really what would the value be of any mechanism designed to reduce the likelihood of some interactions.

Thank you for your attention.

DR. ROBERTS: Thank you, Dr. Kramer. Let me ask the panel if they have any questions or any gentle corrections they might want to offer.

Dr. Hammond.

DR. HAMMOND: I would like to ask whether you have any examples where a coat protein is responsible for a synergistic reaction. Because I can't think of any.

DR. KRAMER: No. Not specifically.

DR. NAGY: Actually, that is -- turnip clinco (ph) virus coat protein is a suppressor of gene silencer.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: I just would like to point out concerning mixed infections that it's generally thought that mixed infections had to do with simultaneous infections.

But just a point of clarification. The infections don't have to occur simultaneously, but, rather, mixed infections represent an accumulation of different plant viruses in the same plant, and those infections, the actual introduction may have occurred at different points in time, which may provide different opportunities for recombination.

DR. ROBERTS: Anyone else.

We're a little ahead of schedule, but we'll go ahead and do the last presentation and then take our

break.

So with regard to the third set of issues then.

DR. MILEWSKI: Thank you. These are the other scientific considerations that I mentioned earlier in my presentation. They are in 2 broad categories, the first one dealing with boundaries of assumptions, and the second one, additional considerations for minimizing risk.

In terms of the boundaries of assumptions, we're seeking your assistance in examining how far VCP PIP can be modified while still supporting assumptions of dietary safety for humans. No new effects on non target species and no potential for novel viral interactions.

In terms of dietary safety, the assumption under which we have been operating is that humans have consumed viral coat proteins for generations as part of the food supply.

Our question would be to what degree and in what ways might a PVCP gene be modified and the PVCP PIP still present no new human dietary exposures. We know that genes can be modified in small ways and large and, for

example, you might even wind up with a gene that is expressing a totally new function.

For us, it is very important to get an understanding of how much modification can occur before you really start seeing something as Mellissa had mentioned earlier, for example, a novel gene, something that is not likely to have occurred in nature before.

And then can the SAP help us with providing a succinct statement describing that boundary?

On the question of no new effects on nontargets, our assumption is that species that interact with non transgenic comparator plants have been exposed to viral coat proteins for generations.

In other words, there would be no new novel exposures. But to what degree and in what ways might a PVCP gene be modified and the PVCP PIP still present no new effects on non target species. Again, we would ask you if you could help us by providing a sussinct statement describing the boundary of that.

Finally, no potential for novel viral interactions. To what degree and in what ways might a

PVCP gene be modified before it becomes a concern that novel viral interactions could occur because the gene could be significantly different from any existing in nature.

And again, can you help us by providing a succinct statement describing that boundary.

Then we're going to ask you if there are any additional risk considerations that we have not touched on in our major group of questions.

For example, are there any considerations related to the PVCP PIP construct that might be considered when attempting to identify risk. For example, does the insertion site have any relevance in considering risk.

Secondly, are there any scientific considerations beyond gene flow, recombination and heterologous encapsidation as posed in the EPA's questions.

Once again, the charge that we're giving to the SAP is to provide scientific advice to assist EPA in its evaluation of several technical issues associated with PVCP PIPs.

Specifically, we're asking you to respond to a series of technical questions related to exposure and hazard considerations for PVCP PIPs.

Those revolve around gene flow, viral interactions and the other scientific considerations which we have just covered.

Now, if you can bear with me, I would like to read into the recorded record the questions that we're going to be posing for you.

The first question is what scientific evidence supports or refutes the idea that plant viruses have significant effects on reproduction, survival and growth of plant populations in natural settings?

Is there scientific evidence that plant populations freed from viral pressure could have increased competitive ability leading to changes in plant population dynamics.

Second question. Please comment on the validity of the agency list of crops that have no wild or weedy relatives in the United States with which they can produce viable hybrids in nature. That is, tomato, potato soybean

and corn.

Question 3. Please identify other crops that have no wild or weedy relatives in the United States with which they can produce viable hybrids in nature, for example, papaya, peanut and/or chick pea.

Question 4. What laboratory techniques used to achieve genetic exchange between species. For example, embryo rescue, use of intermediate bridging crosses, protoplast fusion are not indicative of possible genetic exchange between these species in the field.

Conversely, what techniques, if any, used in laboratory or greenhouse experiments provide the most reliable indication of ability to hybridize in the field.

Question 5. Given that current bioconfinement techniques are not 100 percent effective, what would the environmental implications be of extremely low transfer rates of virus resistance genes over time.

Question 6. Please comment on the prevalence of tolerance and/or resistance to viruses in wild relatives of crops.

Question 7. Please specify techniques that do not or do provide measures of tolerance and/or resistance that are relevant to field conditions.

Question 8. How do environmental or other factors, for example, temporal variations affect tolerance and/or resistance. Given the expected variability, what measures of tolerance and/or resistance would be reliable?

Question 9. What would be the ecological significance if a plant population acquired a small increase in viral tolerance and/or resistance above a naturally occurring level.

Question 10. Please comment on how necessary and/or sufficient these conditions are to minimize the potential for the PVCP PIP to harm the environment through gene flow from the plant containing the PVCP PIP to wild or weedy relatives.

Would any other conditions work as well or better.

Question 11. To what extent are novel viral interactions, for example, recombination, heterologous

encapsidation, involving a viral transgene an environmental concern.

Question 12. What conclusions can be drawn as to whether the likelihood of recombination and/or heterologous encapsidation would be increased or decreased in a transgenic plant compared to its non bioengineered counterpart.

Question 13. How effective is deleting the three prime untranslated region of the PVCP gene as a method for reducing the frequency of recombination in the region of the PVCP gene.

Is this method universally applicable to all potential PVCP PIP constructs.

Would any other methods work as well or better.

Which methods are sufficiently effective and reproducible such that actual measurement of rates to verify rate reduction would be unnecessary.

Question 14. Are any methods for inhibiting heterologous encapsidation or transmission by insect vectors universally applicable to all PVCP PIPs.

Which methods are sufficiently effective and reproducible such that actual measurement of rates to verify rate reduction would be unnecessary.

Question 15. How technically feasible would it be to measure rates of recombination, heterologous encapsidation and vector transmission in PVCP PIP transgenic plants in order to show that rates are reduced.

Question 16. Please comment on how necessary and/or sufficient each of these conditions is to minimize the potential for novel viral interactions.

Please address specifically what combination would be most effective or what conditions could be modified, added, or deleted to ensure that potential consequences of novel viral interactions in PVCP PIP transgenic plants are minimized.

Question 17. To what degree and in what ways might a PVCP gene be modified. For example, through truncations, deletions, insertions or point mutations, while still retaining scientific support for the idea that humans have consumed the products of such genes for

generations and that such products therefore present no new dietary exposures.

Question 18. What are the potential adverse effects, if any, of such modifications on nontarget species. For example, wildlife and insects that consume the PVCP PIP.

Question 19. To what degree and in what ways might a PVCP gene be modified, for example, through truncations, deletions, insertions or point mutations, before it would be a concern that novel viral interactions due to the modifications could occur because the PVCP gene would be significantly different from any existing in nature.

Question 20. Would any additional requirements related to PVCP PIP identity and composition, for example, demonstration that the transgene has been stably inserted, be needed for significant reduction of risks associated with PVCP PIPs.

Question 21. Are there any considerations beyond gene flow, recombination, and heterologous encapsidation as posed in the preceding questions that the

agency should consider in evaluating the risk potential of PVCP PIPs, for example, synergy. Thank you.

DR. ROBERTS: Thank you. Are there any questions for Dr. Milewski on this last set of technical issues of the overall charge to the panel?

Dr. Gendel.

DR. GENDEL: I'm not quite sure how to phrase the question. I'm curious having been on some other SAPs that have considered other PIPs. In question seventeen, you phrase it with the assumption that history of consumption shows that these are safe. How much modification would be necessary to validate that assumption.

In previous cases, we have discussed proteins which also were consumed under various circumstances such as by deliberate application or as contaminants for many generations.

But the agency never phrased the safety questions related to those proteins in the same way. Why are virus proteins being approached differently than other PIPs have been in the past?

DR. MILEWSKI: I'm not sure that we're actually asking -- even though the phraseology may be different, we may not actually be asking a different question.

What we're looking for is the safety consideration in terms of dietary safety of these particular proteins.

DR. GENDEL: You understood my question. In previous SAPs, the question has been actually worded essentially from the other side.

The assumption of safety was not explicit and it was asked how do we establish safety rather than making it explicit here. I just wondered if it was a stylistic thing or there was a reason why this was approached from a different angle.

DR. MILEWSKI: It was approached from a different angle simply because we have a history of comments on the docket which support that assumption. Now, I don't know that I have seen any data in the docket that support the assumption.

DR. GENDEL: Which was the question I asked before. Right?

DR. MILEWSKI: Yes.

DR. GENDEL: Thank you.

DR. ROBERTS: Anything else? If not, let's take a 15 minute break or so then reconvene.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Let's go ahead and reconvene.

Before we move to the public comments section of the agenda, I would like to give the panel one more opportunity if there are any questions or clarifications they would like to pose to the agency presenters before they move on.

Let me just say to preface that there may be some situations where interpretations of the literature by panel members differ from the interpretation perhaps the agency has.

I think the best mechanism to articulate that would be in the context of the questions, the responses to the questions.

So in responding to the question, if part of that involves a different interpretation the agency has taken in the literature, let's highlight it then.

If we get through the questions and there is some literature that we haven't touched upon and individual panel members feel that that it would be important to share a differing interpretation of the literature, let's get to that at the end of the session.

With that in mind, let me ask the panel if there are any clarifications. Other than that, are there any clarifications for the presenters before we move on to the public comment session?

I see none. Before we move on to the public comment session, I would like to thank Dr. Fairbrother, Dr. Milewski, and Dr. Kramer for their presentation. I think that was very useful in terms of helping the panel understand the thinking in the agency and how it has led to the questions that are being posed to the panel. That was very informative for us.

Let's now take public comment. I'm fumbling around to see who the first public commenter is. Dr. John Turner from the United States Department of Agriculture.

And the Animal Plant Health and Inspection Service has requested the opportunity to address the

panel.

Welcome, Dr. Turner.

DR. TURNER: Thank you. I assume my mic is on and you can all hear me.

I am, as you said, with the U.S. Department of Agriculture and Biotechnology Regulatory Services, one of EPA's sister regulatory agencies. But I'm speaking today in the public comment period.

And I thought because this is one of those areas of overlapping authority, one where we have been very active, I offer my comments today just as context. Maybe helpful to know what the other agency, USDA, is doing with respect to virus resistant plants.

We have been regulating virus resistant plants really since the coordinated framework back in 1986. Our original authorities at that time were the Federal Plant Pest Act and the Plant Quarantine Act. Those have since been rolled into one authority, The Plant Protection Act in the year 2000.

That's our authority, the Plant Protection Act. We at APHIS protect plants and animals against all

pathogens and pests. And it is under that authority that we regulate genetically engineered plants. And under the original coordinated framework policy in '86, we had the lead responsible for genetically engineered plants.

There was also some language in 1986 about overlapping authority. When possible, to avoid confusion, it is best when responsibility lies with 1 single agency.

But, of course, that's often not the case. And overlap is always better than gaps. So when more than one agency are involved, there should be a lead agency with coordinated reviews.

This was reaffirmed actually by the NRC, this idea in the 2002 report on genetically modified pest protected plants.

They also said it is good. If there is a lead agency, they stress effective communication and encourage MOUs between agencies to provide guidance for reviews and encourage coordination when there are more than one agency involved.

Under the Plant Protection Act under which we

regulate, the very first piece of text in there really gives you a flavor of our charge from Congress. Congress finds that the detection, control, eradication, suppression, prevention or retardation of the spread of plant pests or obnoxious weeds is necessary for the protection of agriculture, environment and the economy of the United States, and places that responsibility with the Secretary of Agriculture.

And so our regulations under the Plant Protection Act at 7 CFR 340, we have designated genetically engineered plants, certain genetically engineered plants as regulated articles.

And regulated articles are any plants in which genetically engineered plants in which plant pests, any sequences from plant pests are used in the creation of the organism or any transgenic organism where there is a reason to believe that it might pose a plant pest risk.

So clearly, transgenic virus protected plants given issues of recombination, synergy. There are plant pest implications that fit readily into our definition.

So under our authority, we're responsible for

field testing. Virtually any genetically engineered plants, you have to come to us for a permanent or notification. Importation or state movement, same thing, we permit these.

And then after the field testing stage when things are ready for commercial application, one must submit a petition for a determination of non regulated status.

That's where we give our intense scientific review to determine if something is safe for unconfined release.

APHIS has deregulated more than 60 crops representing 14 crop species. Where applicable, EPA and FDA have completed most of these reviews. And many of these have entered commercial production.

This is a list of some of the crops that have been deregulated. You see VR stands for virus resistance. We have had virus resistant potato lines, squash and, of course, papaya.

When we consider something for deregulation, we ask does the organism pose a plant pest risk. Which means

can it cause harm, injury or disease to plants or plant parts.

Also, we ask will the decision to grant non regulated status present a significant impact on the environment. That's to fulfill our responsibilities under NEPA and will the decision have an impact on threatened and endangered species.

In order to make those determinations, we ask for several types of data. We need data on the crop biology itself. Much of the risk assessment relies on understanding the crop biology and especially the reproductive biology of the non transform plant.

Then we look at the genetic differences, the inserted genetic material and its characterization, and we have provided a very detailed guidance on molecular characterization.

We look at any phenotypic differences between the transform plant and a comparator plant. We look at the reports from all of the field tests, any relevant experimental data. And also, applicants are required to give us any unfavorable data or information.

And at the bottom there is a web site, you should have gotten this handout, where you can see all of the data requirements that we have for petitions.

And then analyzing those data we look at plant pest characteristics, generally, can the plant itself cause harm or injury to other plants or the environment, disease and pest susceptibilities, expression of gene products, new enzymes or changes to plant metabolism, weediness and impact on sexually compatible plants, agricultural or cultivation practices, effects on nontarget organisms including humans, effects on other agricultural products and gene transfers to organisms with which it cannot interbreed.

Those are the issues we look at for any crop that comes through deregulation. At this point I'm going to focus a little more tightly on virus resistant plants so you can get an idea of how those have been handled through our system.

First of all, in terms of permits there have been over 850 permits and notifications for field test of virus resistant plants. And because notifications usually

include several sites, you can say that there probably have been well over 1,000 if not a few thousand field tests of virus resistant plants. And there have been 5 virus resistant plants deregulated, starting in December 1994 and the last one in December of '98.

And USDA has also played a leadership role in the organization of meetings to address issues related to virus resistant plants.

I'm not going to dwell on this, but this is a list of the plants that have been deregulated. 2 squash lines there with the virus resistance. The papaya, of course, developed by Dennis Gonzales, and two potato lines.

I'll mention at this point, by the way, I'm not a virologist. If you ask me heavy technical questions about these, I may not know the answer. But my purpose is to give you an overview of flavor for the types of issues that we look at.

So we submitted into the docket for this our decision document an environmental assessment for petition number 9733901, which is a PVY resistant potato. And if

you look through that document, you can see the issues that we addressed in detail.

We looked at plant pest risks posed by these virus resistant plants themselves. Could they somehow cause harm. Plant pest risks posed by the potential appearance of new plant viruses.

In this section we looked at recombination, transencapsidation and synergy. We looked at the potential for changes in weediness or invasiveness of the transformed plant, changes in weediness or invasiveness of the wild relatives, impacts on nontarget organisms including threatened and endangered species and impact on raw and unprocessed commodities.

In addition, we have held several meetings on virus resistant plants. We feel we have played an important role in getting some of these same questions that you are talking about today into the public arena and getting public input and science input into our decisionmaking.

In 1995, in conjunction with the American Institute of Biological Sciences, we at USDA APHIS had a

meeting called Transgenic Virus Resistant Plants and New Plant Viruses. It was actually a workshop. Some of you on the panel were in attendance.

These are the first few questions just to give you a flavor of the types of things, very similar to some of the questions that you are asking, what are the propensities of various taxa to recombine that we consider both between taxa and within taxa.

What are the characteristics of RNA sequences that combine. What data are available on the frequency of mixed viral infections. Is there a difference between the rate of recombination in virus resistant plants expressing a virus transgene compared to plants that express a virus transgene, but are not resistant or compared to plants naturally infected with multiple viruses, getting at that issue of the titer, how important that is, that Melissa Kramer touched on earlier, and how do plant mechanisms such as co suppression that alter the expression of transgene affect the risk of recombination between infected virus and the viral transgene.

I didn't want to make an exhaustive list. Those

are the first questions. There are more questions. They deal with effects of the transgene expression levels on recombination, effect of location of the expression and compartmentalization where you may get expression where a naturally occurring virus wouldn't occur, the likelihood of recombination as a function of the scale of transgene deployment, genomic masking and phenotype mixing, which of course you can get with trans encapsidation, synergy, experimental design and benefits and post commercialization monitoring.

And you can read this report at this particular place on our web. It is still posted.

We held a follow up meeting in 1997 for some new issues. And these are the 4 major areas, not to get into questions.

Recommendations for design of transgenes to minimize recombination concern. There was a section on luteo viruses which really focused more on replicase genes as opposed to virus coat proteins.

Gemini viruses, this was a forward looking section thinking about DNA viruses if they were to come on

and how they might differ from the RNA viruses, and stacking of virus resistance genes.

And that report is also available currently and has been on our web site.

Finally, in 1999, we held with Virginia Tech's information systems for biotechnology a workshop on the ecological effects of pest resistance genes and managed eco systems. So it wasn't specific to viruses, but there are many instances in there where we did discuss viral implications.

That's when we really got into weediness, fitness characteristics and gene escape. And we looked at the potential impacts of the weediness of these crops, gene escape, potential weediness of wild relatives, and, then, of course, the important question is the role of pathogens in limiting weed populations.

Gene flow per se is not a risk if there is no impact. But if it gave resistance to a pathogen which was limiting a population, that would be significant. And we talked about gene stacking and crop specific parameters, which could affect impacts.

And so these types of meetings are an ongoing event for USDA APHIS.

Finally, in summary to pull all this together, transgenic virus resistant plants clearly meet the definition of regulated articles based on being derived from plant pests and their potential to pose a plant pest risk.

It will continue to be a central activity for USDA APHIS as is our charge from Congress under the Plant Protection Act.

We have a long history of regulating transgenic virus resistant plants through the permitting of field tests. The first one was in 1988 with the virus resistant plants.

And we consider many of the big issues that have been discussed at these previous workshops and are on your agenda of questions today, virus recombination, trans encapsidation, synergy and weediness and fitness of the transgenic crop and wild relatives.

And we will continue to be on the forefront in raising questions for virus resistant plants and gaining

outside input to enhance the effectiveness of our regulation.

I hope this has given you -- your charge is with EPA. This is purely what we at USDA do. I hope it has been helpful for you. Thank you.

DR. ROBERTS: Thank you, Dr. Turner. Are there any questions from panel members regarding Dr. Turner's presentation, the regulatory role of USDA?

Dr. Hammond.

DR. HAMMOND: Yes. How does APHIS view the regulation of cross protection the deliberate inoculation of crops with a mild isolate virus to protect against the effects of a severe isolate compared to the use of transgenic plants expressing coat proteins?

DR. TURNER: Well, with the isolate, the mild isolate, if it were genetically engineered, it could likely be a regulated article. And if it were, then we would be regulating that.

Can you give me a little more feel for in terms of how you regard it? I mean, it is our role, of course, to see that field tests are safe and deregulations are

safe and not really move one technology or the other forward.

DR. HAMMOND: I was essentially getting at whether there is concern about the use of cross protection as compared to transgenic plants expressing coat protein because both are doing the same thing to a large extent.

You have a deliberate presence of virus coat protein in the crop plant.

DR. TURNER: Right. And I don't know if I'm prepared to answer that in that I'm not familiar with any review that we have done of the cross protection in terms of the issues that would be raised versus transgenic plants.

So maybe I don't have a good answer for that. But certainly, if it were using viruses which occurred in that area and didn't pose a new risk and it provided some sort of protection, it would be something which it seems as though the risk issues could be addressed in some suitable way.

DR. ROBERTS: Any other questions? Thank you very much, Dr. Turner, for joining us in

clarifying those issues.

The next public presenter would be Dr. Susan Tolin.

Welcome, Dr. Tolin. Could you introduce yourself for the record.

DR. TOLIN: Yes. I'm Dr. Sue Tolin. I'm commenting today on the behalf of the American Phytopathological Society or APS.

In that capacity, I represent our approximately 5,000 members who work with plant pathogens and the diseases they cause. In that work, they device ways of managing losses that are caused by plant pathogens.

I have been active in the society serving as president, about 10 years ago, and as a member of the public policy board until just 2 years ago. And in this capacity I have had experience in addressing the issues that are important to plant pathology, including the regulation of biotechnology.

I'm also a plant virologist by profession and a professor at Virginia Tech. What I will comment on today, though, is APS's activities, because we have

gone on record as supporting the use of biotechnology as a means of improving plant health, food safety and sustainable growth in plant productivity.

In 2001, APS issued a position statement on biotechnology, and I have given reference in the written text to the APS web site where you can see this complete statement. But I'll pull out a couple things that are relevant to today's discussion.

The first is that we stated that insertion of viral sequences into the plant genome to cause plants to resist virus infection provides a new and effective genetic approach for managing plant viruses. Secondly, future environmental benefits of biotechnology for improved management of plant diseases are enormous. Particularly, the potential to reduce the dependency of growers on synthetic pesticides and to enhance approaches that minimize adverse effects to the environment.

And then the concerns that are being raised of environmental and food safety risks of biotechnology through, first, gene exchange or, second, evolution of plant pathogens, or from putative increased or unexpected

allergenicity are legitimate risks that will be addressed as have similar potential risks with any new plant or plant product.

Assessment and management of these risks and other risks of new technologies in the form of process is appropriate and must be conducted in a science based manner, and
also consider economic, human and animal health and ecological consequences.

The statement went on to say that the risks and concerns of plants modified through biotechnology must be viewed in perspective relative to other genetic modification methods and that the consequences of not using biotechnology as an augmentation over a controlled methodology must also be considered.

For many years, APS has followed the issue of regulation of biotechnology by federal agencies. During my presidency of APS, EPA first proposed its policy to define substances produced in plants that play a role in resistance to pathogens as plant pesticides. And therefore, is subject to regulation under FIFRA.

At that time APS provided comments to the 1994 Federal Register on this proposal, and included our support of the exemption of coat proteins from plant viruses that was proposed at that time.

APS was instrumental in developing a report in 1996 from a coalition of 11 scientific societies, which examined the scientific basis for EPA's proposed policy, and actually prepared a report that was called Appropriate Oversight for Plants With Inherited Traits for Resistance to Plant Pests.

In this, there were provided principles for oversight that are currently used in plant breeding and cultivar release that is done in a non regulatory fashion and has been used for plants prepared by conventional methodology.

And this is available on a web site which is included in the written document.

We continue to dialogue with EPA ultimately leading to a change in the name from Plant Pesticides to Plant Incorporated Protectants which is currently used.

APS also provided extensive comments to EPA on

the 2001 proposal for PIPs, and these are posted on our web site. At that time we continued our support for the full categorical exemption of the plant virus coat protein.

Relative to the specific comments and the charge to the panel, we feel that the charge that we have heard this morning has been derived from comments received in these prior publications in 1994 and 2001 that have highlighted the areas of greatest scientific uncertainty.

The questions asked of the panel should enable the panel to discuss the deployment of coat protein to protect plants from viruses and to explore whether scientific information gained in recent years can be used to decrease the level of uncertainty of environmental impacts.

The background material and literature review provided are adequate, but there are still many unanswered questions, simply because the research has not been done and little research funding has been directed to these areas.

The panelists for this meeting, many of whom are APS members, certainly have the accumulated expertise to provide an excellent review for EPA. Thus I will not attempt to provide any additional indepth response from APS to all the questions that are answered, but simply to bring up a few points that were made in our prior comments. Regarding the question of gene flow as an environmental hazard and its possible mitigation, we concur with the conclusion that gene flow can occur from plants containing PVCP PIPs to wild or weedy relatives.

Molecular and genomic approaches have provided the tools to demonstrate that gene flow is probably much more extensive than we previously realized. But the interpretation of the consequence of this is still in its infancy.

Gene transfer alone, however, should not categorically be considered an environmental hazard, but a natural process. There is little information on flow of resistance genes from crop plants to wild or weedy species and whether or not that has ecological implications.

Specifically, could virus resistance confer a

selective advantage on wild or weedy plants.

Many weeds are symptomless carriers of viruses that commonly infect crop plants and do not appear, at least in my observations, to be adversely affected relative to their population and geographic range. But I'm sure we'll hear more on this from the panel.

As I said, weeds are reservoirs for virus inoculum. Thus, if we transferred natural resistance gene to the weeds, this could actually help reduce virus reservoirs while having little or no effect on the weed.

Crop plants are often developed by conventional breeding to be resistant to viruses because this is a major constraint in productivity.

Yet, there is no evidence that I'm aware of that such resistance genes have moved from the crop plants to wild species. In some cases, but not as often for viruses as for other pathogens, wild species have been the source of resistance genes.

Many of these points were discussed in the 1999 workshop on ecological effects of past resistance genes and managed ecosystems that Dr. Turner just mentioned.

And we suggest that EPA look at this document.

Mitigation of gene flow could be accomplished simply by spatial and temporal separation of the species. The species have to be together and they have to flower at the same time for gene flow to occur.

Concerning mitigation by risk management, in our 2001 comments to EPA, APS strongly supported the position that the review by USDA that Dr. Turner has just described, concerning gene flow, that this was sufficient regulatory oversight of this potential risk.

We trust that EPA as it explores this area further or takes further action on it will continue to work cooperatively with USDA.

On the second charge, do viral interactions pose environmental hazards and could they be mitigated, we recognize that the potential exists for any viral transgene to recombine with viruses infecting the transgenic plant and that recombination to form new viruses or virus strains can occur in certain circumstances.

New virus emergence per se does not pose an

environmental hazard. The phenomenon of new virus appearance during mixed infections or increased virus diversity as influenced by its host or vectors is known to occur in nature.

Such phenomena are much more readily demonstrated today with the increased knowledge of viral sequences and the tools of viral genomics and bioinformatics. The significance of this emergence could now be explored if more funding were available.

The panel will undoubtedly bring up many specific examples. I look forward to listening to their discussion during the course of this meeting.

With regard to the other questions, in its 1994 comments, as I said, APS supported the exemption of viral coat proteins and the tolerance, level in the tolerance level.

As of that time, there was no known toxicity or allergenicity of coat proteins to humans. We were concerned at that time of possible modifications to the proteins and made the statement that APS suggests additionally that the language in the exemption to

tolerance requirements should be made perfectly clear to refer only to those viruses normally infecting plants.

At that time, we were aware that research had just begun to modify coat proteins to express, for example, epitopes from animal or human viruses which specifically we believe should not be covered by this exemption, but should be examined more completely.

The mechanism of synergy between viruses was largely unknown in 1994, but today it is quite well understood as a function of certain portions of the viral genome in gene silencing and silencing suppression.

To summarize, APS supports the exemption of the application of plant virus coat proteins incorporated as protectants for the control of plant virus diseases.

Assessment and management of risk must be conducted in a science based manner and should also consider the benefits resulting from deploying these resistant plants.

Risks should be viewed in perspective relative to other genetic modifications and virus control methods.

Thank you for the opportunity to present these

written and oral comments on behalf of APS, and I will be pleased to answer any questions.

DR. ROBERTS: Thank you, Dr. Tolin. Do any panel members have any questions for Dr. Tolin, her presentation? I don't see any. Thanks very much.

I would like to also point out for the audience that there have been a number of written comments provided by interested parties for public comment regarding today's session.

Those written comments have been copied and distributed to the panel members and they are also available for public review on the docket.

At this time I would like to ask if there are any members of the audience who would like to make comments to the panel on this topic.

In other words, is there anyone who has not previously indicated a desire to address the panel on this but would like to do so now? I would point out that this is really the only opportunity in the agenda for this meeting for public comment.

I don't see anyone. In that case, let me thank,

then, Drs. Turner and Dr. Tolin for coming here, making presentations to the panel. We appreciate that. Appreciate the information that you have provided.

And also thank the other folks who were not able to make presentations, but provided written comments for the panel. The panel takes very seriously input from stakeholders and the public in our deliberations on these issues. And we appreciate the effort that was expended to make that information available to us.

This, then, closes the public comments section portion of the agenda. It is 11:15. I think that we have time for the panel to maybe tackle the first question on our list. Get one under our belts before we go to lunch. So let me suggest that we do that.

Can I ask the agency to pose the first question to the panel.

DR. KRAMER: What scientific evidence supports or refutes the idea that plant viruses have significant effects on reproduction, survival and growth of plant populations in natural settings? Is there scientific evidence that plant populations freed from viral pressure

could have increased competitive ability leading to changes in plant population dynamics?

DR. ROBERTS: Dr. Sherwood, would you lead off our discussion in response to this question.

DR. SHERWOOD: We have been asked to begin this session on gene flow with a discussion of what scientific evidence supports or refutes the idea that plant viruses have significant effects on reproduction survival and growth of plant populations in natural settings. Is there scientific evidence that plant populations freed from biopressure could have increased competitive ability leading to changes in plant population dynamics.

Agro ecosystems are not natural settings. Even if we look at our production areas at a larger scale, beyond the borders of a field or fields, we are still examining an environment that has long been disturbed.

If we were to examine natural settings, I'm not aware of any extensive inventories of plant virus in an undisturbed ecosystem.

As viruses are obligate parasites, it would be an evolutionary dead end if they impacted their host or

vectors too significantly. Thus for the sake of the initial comment for this session, I'm considering the natural setting as those areas adjacent to production areas.

Our primarily knowledge about the effects of virus on plants come from cultivated plants. And a trite but truthful answer to the effect of plant viruses on reproduction, survival and growth of plants is it depends.

The obvious goal with cultivated plants is to lessen the impact of virus infection on plant grown and subsequent yield of the plant part of commercial interest.

The effect of virus infection on cultivated plants is quite variable and dependent on a specific host and specific virus.

Microscopic symptoms can include reduction in growth, reduction in vigor, reduction in quality or the infection may be masked.

While we do know something about the impact of virus on cultivated plants, our knowledge about the effect on virus infection on non crop plants is quite limited

and, again, could be succinctly answered, it depends.

Most virus epidemics result from the virus and or vectors coming from non crop plants adjacent to production areas. If the host from these natural settings are too adversely affected by the virus or vector, the epidemic cycle would be broken as the plant reservoir for virus and vector would no longer be present.

As summarized by Duffus, from the standpoint of control of virus diseases, there is perhaps no phase of virology more important than epidemiology.

The role of weeds in the occurrence and spread of plant virus disease is an integral part of the ecological aspect of virus transmission.

So the question now becomes what is the impact of viruses on weeds. The literatures is filled with reports of different viruses on different plant hosts either found in natural infections or purposely inoculated as plant host strains has long been a method to differentiate viruses and virus strains.

What is lacking is a significant body of literature on the effect of viruses on weed species.

Freiss and Maillet found that in cucumber mosaic cucumo virus infected chick weed plants, stellaria media, grown in a monoculture had similar vegetative production to a monoculture of control healthy plants.

However, when healthy and infected plants were grown together, as the density of the healthy plants grown with infected plants increased, infected plants were not as vegetatively productive or as reproductive.

Work from this lab on nitrogen partitioning and CMV infected versus healthy weeds found no difference in virus infected and healthy chick weed plants, but nitrogen partitioning to shoots and roots was different in CMV infected and healthy purslane, portulaca oleacea.

Romold examined the incidence of barley yellow dwarf luteo virus in 3 grass hosts, soft brome grass, green foxtail and yellow foxtail.

Using panicle length as a measure of fitness, soft brome grass was not affected by virus infection.

Fitness of green foxtail was about half of uninfected plants. And infected yellow foxtail had about 25 percent greater fitness than uninfected plants.

Maskell, et al., found that wild cabbage inoculated with either turnip mosaic poty virus or turnip yellow mosaic tymo virus had significantly reduced survival, growth and reproduction.

In a recent 3 year study of CMV in central Spain, Sacristan found that the incidence in CMV in weeds fluctuated in various habitats such as fallow fields, edges and waste lands through the growing season with a maximum incidence of 20 to 30 percent in summer and autumn. The greater amount of biomass and soil coverage was correlated with a greater incidence of CMV.

Thus, there is quite a bit of variation in the impact of viruses on plant growth. Virus infection, regardless of the plant being a crop or a non crop plant, will likely negatively impact some aspect of plant development and reproductive capacity.

Is there scientific evidence that plant populations freed from viral pressure could have increased competitive ability leading to changes in plant population dynamics as the second question posed.

I'm not aware of any study that has purposely

freed a weed species of a known virus and determined its competitive ability or looked at the population dynamics of virus infected versus uninfected plants in a multiple species ecosystems.

However, if we were to use the definition of a weed as a plant out of place, our agro ecosystems provide many good examples as most crop plants grown in tempered ecosystems do not originate in those ecosystems.

The classic example is running out in potato that results from accumulation of pathogens, particularly viruses, in the vegetatively reproduced seed material versus the use of true seed that is commonly used for most crops.

Virus free seed potatoes, developed through certification programs, are far more vigorous than virus infected seed potatoes.

For viruses that are transmitted through true seed, greater vigor and reproductive capacity is also commonly observed for plants originating from virus free seed.

Starting virus free, regardless of the plant

being a crop or non crop species does not give a plant immunity.

Weeds or crop plants that are susceptible to viruses in that environment can be infected.

We see from the work of Remold cited above that viruses have variable effects on non crop plants.

In regards to plant population dynamics, the effect of tomato spotted wilt tospo virus on peanut plant production is a good demonstration of the effect virus can have on a population dynamics.

Fields of peanut plants that have no or little virus are more competitive in that the vines lap earlier, blocking the sun between rows, thus reducing weed pressure.

The significant study of Jones and Nicholas in self regenerating pasture in Australia over a four year period provides a good look at the introduction of virus in a multiple species complex environment.

They sowed seeds of burr medic that was either free or infected with alfalfa mosaic alfamo virus in mixed species pastures and followed the effect on proportionate

of species over time.

Generally, less desirable species became established as the virus became established, but the effect varied with medic cultivar.

Difficulty in determining the effect was compounded with the extent of aphid abundance that transmits AMV which was variable. I'm not aware of any reports on the impact of virus infection on plant competition in non agricultural settings.

DR. ROBERTS: Thank you, Dr. Sherwood.

Dr. Cooper, would you like to contribute to the response?

DR. COOPER: I will add only a few things to what John said. Of course, the impact is strongly suspected, but not proven. We have quite a lot of evidence of impact upon wild plants, but many of those wild plants are not known to be crucial to the survival of the virus in its evolutionary sense.

It may have many alternate hosts. So we can't make definitive judgments about whether devastating effect of one particular species would really have any serious

impact. It may have several alternative ways of using it.

All virus isolates are not equal. And all, of course, of the plants that we group together as species are not equal in terms of the reactions to the viruses.

Fundamentally, we don't have very much information, if any, about the whole life cycle impact of any of these viruses.

Dramatic impact on seed production, growth, vigor, which we can recognize, is not felt to be an adequate description of what the impact would be on an evolutionary sense on the species.

And so what we ideally seek is some life cycle assessment over the stages which are crucial to the survival of the species from seed back to seedling, seedling to flowering plant, flowering plant to seed, including what proportion of the seeds are lost through whatever cause.

And those together make the population dynamic of a wild species, which is perhaps a somewhat different sort of approach to that which might be considered by in an agricultural context.

Because those pieces of data are absent, it is really difficult to prove the concept which is implicit in the question. So that we have differential virus patho (ph) types with different effects and we don't know the full life cycle implications of any of them that we have observed so far.

DR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: I think my colleagues have stated the case.

DR. ROBERTS: Let me then open the question up to other members of the panel, their viewpoints that they want to contribute, agree with, disagree with, so forth.

DR. FALK: I agree with the comments that have been made previously. I think one of the things that we're hearing in regards to these questions is the assumption that or it is based on the assumption that viruses are pathogens.

I think it is legitimate questions (ph). They are viruses (ph). Are they naturally pathogens with their natural host plants or even host animals.

If they are not pathogens, then in a natural

system, then, some of these questions are not that relevant.

I too am not aware of data that shows that plant viruses have significant effects on characters in plants in natural settings.

There are some reports, however, that show the opposite. For example, a report by Adrian Gibbs shows that virus infection in wild legum host actually protects that plant against herbivore by some animals that eat that plant.

In some cases, virus infection in their natural host can actually confer advantages. So they are not always pathogens.

I think if viruses had obvious negative effects on wild hosts, this could have been noted already or we should have noted this.

I think that plant viruses and all viruses do not necessarily kill their host plants. We see serious effects on our cultivated crops and the losses that we see are those that Professor Sherwood mentioned. I think that if we think of virus disease, we have to

consider population and inoculum pressure. In the past we have controlled viruses through many means.

And when we control the viruses in agricultural settings, if those viruses were important in affecting weeds in a natural setting, we have already reduced inoculum and should have seen some effects.

So I don't think or my point is I'm trying to bring up the or have us think about whether, in fact, viruses actually are controlling weeds in natural settings.

DR. ROBERTS: Other viewpoints. Dr. Tepfer.

DR. TEPFER: I just wanted to sort of propose a type of clarification, which is quite in the same lines of what the other panel members have said.

I think that it is very important to make the distinction between the effect of a fitness advantage in which case you could expect that a virus resistance transgene would become more and more frequent within a population of a wild or weedy species and that is a quite different situation from actually having effect on the size or distribution of the populations of the plant

species in question.

It is only the latter situation which would constitute ecological release and could increase weediness.

As the other speakers have said, there are numerous reports of changes in fitness effects and because these are experimentally relatively manageable.

But I think that doing an experimental study on ecological release is remarkably difficult because in many cases you can have a fitness effect which may not be limiting to population size.

DR. ROBERTS: Any other comments from panel members. Yes, Dr. Hammond.

DR. HAMMOND: I just would like to say I pretty much agree with what has been said so far, but I would like to further go and document the fact that there are frequent occurrence of mixed infections of viruses in wild plants without obvious evidence of any symptoms or deleterious effects.

In a survey that I carried out myself as part of my doctoral research, I looked at viruses infecting the

common weed plantago lanceolata.

And randomly collected plants from wild settings around the country without regard to any symptom expression and found that seventeen percent of these randomly selected plants had, in fact, multiple infections with as many as 4 viruses present in a single plant without any obvious deleterious effects.

This has been documented in other species as well. Alan Dodds carried out a study in nicotiania glauca and found that infections of 5 to 7 viruses in single plants were common again without significant apparent obvious effects on the plant.

And Jim Duffus had found up to 9 viruses in individual plants of spinach. There are also interactions between viruses and other pathogens, in some cases positive effect and in some cases negative effects.

One that comes to mind is an effect between barley yellow dwarf infection in some grass populations having a protective effect on a fungal disease that otherwise infects those plants.

But there are also cases where virus infection

increases susceptibility to fungal diseases.

So there are many cases when wild living plants are infected with more than one virus without any apparent detrimental effect.

DR. ROBERTS: Other questions from panel members.

DR. KRAMER: Can I ask for one clarification. What I have heard from a lot of the panelists is that there is basically a lack of evidence suggesting this changes in plant population dynamics.

Would you consider that sufficient to conclude that it does not occur or are you saying that there simply is no evidence to conclude either way?

DR. ROBERTS: Dr. Cooper and then Dr. Sherwood.

DR. COOPER: If there is no evidence, then there is no evidence on which you can make an assumption. At the moment it's being investigated. One specific example, brassica rapa and now it's called compestris. That's been initially resulting in evidence that suggests the impact on seed production is not crucial to the survival of the

species.

But there are several more years worth of investigations even in that species in relation to one virus, turnip mosaic virus. And even that might not be generally applicable, but it would at least answer all the points which seem relevant to the survival of the species, its persistence and it's dynamic.

In the absence of those data at the moment, I would strongly recommend that we shouldn't rush to judgment.

DR. ROBERTS: Dr. Sherwood, do you want to add anything?

DR. SHERWOOD: I think he said it far more eloquently than I could have.

DR. ROBERTS: Any other follow-up questions or clarifications?

Let me poll the panel members. Do you want to go ahead and take the second one or do you want to break a little bit early for lunch and then come back?

DR. STEWART: Take the second.

DR. ROBERTS: We have a vote to take the second

from the lead discussant on this. Let's go ahead and take the second question.

DR. KRAMER: Number 2. Please comment on the validity of the agency list of crops that have no wild or weedy relatives in the United States with which they can produce viable hybrids in nature. That is, tomato, potato, soybean and corn.

DR. ROBERTS: Dr. Stewart, would you lead off our discussion on this one.

DR. STEWART: This list is adequate insofar that it lists crops of large acreage in the U.S. I think it is dandy.

DR. ROBERTS: Well and concisely stated. Dr. Cooper.

DR. COOPER: I would question one of the species in this list, is tomato. To my mind, it has a strong potential at least to be a weed.

In U.K. conditions, it is manifestly a nuisance plant in the vicinity of sewage treatment works because the seed of tomato grows readily through the human and indeed the rodent elementary canal.

So the transmission of the seeds of that species into places of accumulation and disturbance results in lots of opportunities for tomato.

Tomato doesn't invade agricultural land, but it is certainly a nuisance plant which at least in British conditions is recognized as such with the potential. Perhaps in other parts of the world.

I don't know about the American experience, but it does seem to me worth flagging that difference.

I think cuba bearing salinums (ph) are well established as being rigorously isolated from one another, and, therefore, even if any of them were nuisance plants, as sometimes they can become, the risks of moving genes between them would be minimal. Perhaps even non existent.

But some of the others I don't know about. I can defer only to the local expert. DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I have nothing to add. I agree with Dr. Stewart.

DR. STEWART: I don't think tomatoes is naturalized in the U.S. I think the U.K. experience is a

bit different there. DR. ROBERTS: For the record, that was Dr. Stewart.

Dr. Tepfer.

DR. TEPFER: I just have a question, in fact, of clarification regarding this list. Does this include also territories associated with United States that are not the 50 states, some of the tropical territories as well?

DR. KRAMER: Yes. If I remember correctly, I think there is a footnote in the background paper that we handed out that would list all of the included territories in the statement.

DR. ROBERTS: Other comments or comments from other panel members on this?

Was the response from the panel concise and clear?

DR. KRAMER: Yes.

DR. ROBERTS: Taking the direction from Dr. Stewart, I suspect we could probably take number 3.

DR. STEWART: Number 3 might take a little bit longer, but I'm willing to go at it if you are.

DR. ROBERTS: Let's do number 3. We're on a

role.

DR. KRAMER: Please identify other crops that have no wild or weedy relatives in the United States with which they can produce viable hybrids in nature, for example, papaya, peanut and/or chickpea.

DR. ROBERTS: Dr. Stewart.

DR. STEWART: This question depends on which crops are grown, where they are grown in the U.S. Annual crops can go year to year, deployed in time, whereas perineals can be long lived. Crops can be also naturalized and considered wild or feral at some point.

So the question pertains to wild relatives per se and not whether the crop will hybridize or introgress with them. We're looking at this pretty broadly. And we're looking to exclude plants that we can maybe move up into question number 2. The list in number 2.

And I think these could be considered candidates (ph).

And I'll be interested in hearing what you all think of my list anyway. This is the one time which I had my university ovarium curator in my back pocket.

So the list of crops without wild relatives in the U.S. that I'm aware of would be papaya, peanut, chickpea, bean, pea, black eyed pea as we say in the south, cow pea other places, lima bean, cucumber, sugar cane, onion, pepper, spinach, barley, peach, almond, citrus, sweet potato, daffodil, olive, and I have question marks beside chrysanthemum, tobacco and apple, the last 3.

Tobacco is an American -- it is indigenous to the Americas. I'm not sure how many wild tobacco there actually is left in the U.S. I don't know.

You know, there are new crops coming up every year. I guess this would be the larger crops and no one really would consider daffodil to be a large crop. So there is that caveat.

DR. ROBERTS: Let me just ask did everybody get a chance, since this is a good starting place for discussion, do you need Dr. Stewart to go through the list again or did everybody get them down?

Do them one more time.

DR. STEWART: Papaya, peanut, chickpea, bean, pea, black eyed pea or cow pea, lima bean, cucumber, sugar

cane, onion, pepper, spinach, barley, peach, almond, citrus, sweet potato, daffodil, olive, and chrysanthemum, tobacco and apple.

One of the commenters also included brassica oleracea vegetable such as cabbage, cauliflower, broccoli, those types of things. And I wasn't as comfortable with that one because there are wild relatives that share a genome from brassica oleracea even though they don't cross very easily. DR. ROBERTS: Dr. Cooper, what do you think about list.

DR. COOPER: It is very long. At least the brassica having seen wild or perhaps feral brassica oleracea types in San Francisco just across the Bay reasonably abundant, I'm surprised -- they may not be a weed situation, but they are reasonably abundant in the wild.

So at least they may be isolated physically from many potential transgenic crops, but nevertheless I consider even in my limited experience that they are there.

I really can't comment about most of the other

things. The tobacco is such a variety of different types. When we looked at the risks of gene flow into tobacco, many of the ornamental tobaccos were actually thought to be on limited experience genetically isolated from the nicotiania tobacum types we were using. But the evidence was not a complete basis for making a safety judgment.

I won't comment on anymore at the moment.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I'm not aware of any wild relatives. I don't consider myself qualified to judge in this area.

DR. ROBERTS: Let me ask other panels members if they want to weigh in. Dr. Melcher.

DR. MELCHER: I would like to have it clarified for me not being a taxonomist what is meant by a relative in this case. Because I can recognize that some of these are legums and I know that there are wild legums and some of them are rosacea. There are wild rosacea and so forth.

DR. STEWART: My interpretation of this question

takes it to the species level in many instances, the general level in some instances. That is my criteria for not including some things on this list. And then relative abundance.

So there is a lot of interpretation here. If we get right down to brass tax, all the plants are related at some level. So you would have nothing on the list.

DR. ROBERTS: For the record, that was Dr. Stewart responding. Let me go ahead and ask the agency to clarify that for us.

DR. KRAMER: I want to clarify. I think what we're concerned with is the latter part of that sentence where we're saying can produce viable hybrids in nature. So how ever you would define relative that would encompass such plants would be fine.

DR. ROBERTS: With that in mind, you're still comfortable with your list, Dr. Stewart?

DR. STEWART: Yes. That's how I interpreted this list. These would have an extremely low chance of forming viable hybrids.

Now, you could really add more plants to this

list where the hybrids would be really low fertility or the hybridization rates would be extremely low.

Especially, when you consider where the wild and the weedy relatives might be compared with where a crop is grown.

Here again with annual plants, the crop can be grown in different places each year. With perennials, they are a little bit longer term.

DR. ROBERTS: Dr. Zaitlin and then Dr. Tepfer.

DR. ZAITLIN: I was going to say with tobacco there are a number of nicotiania species that do grow wild in the south western United States, but I think genetic incompatibility with nicotiania tobacum.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I want to confirm and remind you that the commercial tobacco, nicotiania tobacum, is an allotetraploid. And is in fact genetically quite completely isolated from anything that grows in the United States.

And the tetraploid form does not grow in nature. It has never been described, and the two parental species

come from very obscure places in South America. I think tobacco is one we could definitely add to the list.

In contrast I'm a little bit concerned about pepper, which is capsicum because there are feral populations of capsicum in Caribbean Islands.

So that might be one that we might put a question mark behind at least. I suspect that you could get gene flow in places like Puerto Rico and things like that, virgin islands.

DR. STEWART: When it comes down to I guess the really tropical locations, territories, Hawaii, granted I think I need to do some more study there, the list is a bit shaky.

My list is mainly pertains to continental U.S.

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: I just wanted to apologize. The footnote I was referring to disappeared in a draft. It was unintentional. I wanted to read into the record what exactly we mean by the United States in this context.

That would mean a state, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin

Islands, Guam, the Trust Territory of the Pacific Islands, and America Samoa.

DR. STEWART: What was the last 3?

DR. KRAMER: American Samoa.

DR. STEWART: What was before that?

DR. KRAMER: Let me read the whole list again.

A state, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, the Trust Territory of the Pacific Islands and American Samoa.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I would just raise the question about sugar cane because again there is a lots of feral sugar cane in many tropical islands. I'm not sure how sexy it is. I think that a lot of sugar cane is rather sterile. I think you can make crosses.

DR. STEWART: There is a shatter cane that's actually compatible. I was not considering the Caribbean when this came up. That one should perhaps be removed from my list anyway.

DR. ROBERTS: When we write up our minutes,

maybe we can sort of clarify which ones we have high confidence in and which ones given the other territories that we're talking about a little more far flung that we might have some reservations about.

Any other comments, edits to the list?

Dr. Kramer, is that response do you think going to meet the needs of the agency?

DR. KRAMER: I would ask when you are writing up the final minutes to indicate whether this is a consensus view. I know you had expressed some reservations about the list at this point. We ask in the final write up to be sure that you are comfortable with the list that you actually put in the minutes.

DR. ROBERTS: Let me ask right now while we're in session. Is everyone pretty comfortable? Let me ask it this way. Is there anyone that is uncomfortable with the list, with the caveats that we anticipate about some of the territories?

DR. COOPER: I'm not comfortable with the brassica.

DR. STEWART: Brassica wasn't in there. As I

noted, a couple of the commenters included brassica oleracea vegetables. My list did not have them for the very reasons that you mentioned.

DR. ROBERTS: So there seems to be agreement on that. That that probably shouldn't be on the list. Dr. Tepfer.

DR. TEPFER: I don't seem to have the list, the preexisting list. Could we leave this open until after lunch so I can have a look at the previous list to see whether there are things that strike me on it before we come to a final conclusion on this point?

DR. ROBERTS: Sure. There is no problem with that.

Then we are getting kind of close to lunchtime. Let me then suggest that we take a break now. We will close out this question when we return from lunch. Let me suggest that we do that at 1 o'clock.

So I'll give you guys a chance to sort of take a look at that list. We'll finish up question 3 immediately when we convene at 1 and then we'll proceed on with question 4.

Let's plan on getting together back here at 1 o'clock.

(Thereupon, a luncheon recess was taken.)

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I N V O I C E

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